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THE MOLECULAR ARCHITECTURE OF FOCAL ADHESIONS

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CONTENTS

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ABSTRACT

This article outlines the present knowledge of the architecture, molecular composition, and dynamics of focal contacts of adhesive animal cells. These structures, developed at the plasma membrane at sites where cells touch their substratum, are essential for cellular attachment in tissue formation during embryogenesis and wound healing. In tissue culture, they are particularly prominent and thus amenable to detailed investigation. Focal contacts consist of a cytoplasmic face, comprising cytoskeletal elements, a transmembrane connecting region, and an extracellular face composed of proteins of the extracellular matrix. The molecular anatomy of the numerous proteins involved, the basis for classifying them as structural or regulatory components, and their in vitro interactions are described. Based on this information, current models on the dynamics of their assembly and of possible regulatory mechanisms involving a variety of signal transduction pathways are discussed.

INTRODUCTION

Tissue formation in animals that takes place during embryogenesis, metamorphosis, and wound healing depends critically on the ability of many cell types to form specific contacts with each other and with the extracellular matrix. These contacts are defined by morphologically discrete structures developed at both sides of the plasma membrane. The core of these structures comprises transmembrane proteins that mediate between the intra- and extracellular regions. Their cytoplasmic domains connect with cytoskeletal elements, whereas their extracellular parts are engaged in making contact with elements of the extracellular matrix or of neighboring cells. Although sufficiently rigid to guarantee firm cellular adherence, both types of contacts are also highly dynamic and can be reversibly assembled and disassembled within minutes. This is initiated by a response to either cytoplasmic or external signals.

Although cell-cell contacts can be further divided into different types of junctions classified by junction-specific proteins, subclasses of cell-matrix junctions are not as easy to define. Using criteria such as life span, size, and morphology, and studying cultivated adhesive cells, one can discriminate between small, dot-like spots where mobile cells adhere transiently to their matrix ("podosomes;" Tarone et al 1985, Gavazzi et al 1989) and the large "focal adhesions" (Abercrombie et al 1971), which often last for hours or days, as observed in cultures of epithelial, endothelial, or fibroblastic cells. Typical focal adhesions are mainly developed in tissue culture and are only rarely found in the organism (for example, in cells subjected to shear stress; cf Byers & Fujiwara 1982, White et al 1983, Gabbiani et al 1983, Hüttner et al 1985, Drenckhahn & Wagner 1986). However, because tissue culture cells are ame-

nable to microscopic and biochemical analysis and can be experimentally manipulated, they have proven an attractive object for the analysis of architecture and regulation of cell-matrix contacts in general. In addition, the attachment plaques developed by activated blood platelets are considered as a model for focal contacts (cf Nachmias & Golla 1991, Fox et al 1993), as well as for the dense plaques of smooth muscle cells, and the myotendinous junctions of skeletal muscle (see Burridge et al 1988 for references).

Initially, focal adhesions were described in living or fixed fibroblasts grown on glass or plastic, as seen by reflection contrast microscopy and electron microscopy (Abercrombie 1971). They consist of a spear tip–like structure, up to 10 μm in length and 0.5 μm in width, connecting the extracellular matrix with the ventral plasma membrane (Figure 1; Abercrombie & Dunn 1975, Izzard & Lochner 1976, Heath & Dunn 1978). At the cytoplasmic face, these structures correspond to tightly packed ends of microfilament bundles in the terminal portion of stress fibers (Figure 2). Our present knowledge of the molecular composition of these regions is mainly based on immunochemical and biochemical analyses. Indirect immunofluorescence with specific antibodies has been widely used to identify individual proteins as components of focal adhesion sites (cf Figure 1*d*). Although indirect immunofluorescence on fixed, detergent-extracted cells certainly cannot be considered an ideal technique to elucidate the native distribution and concentration of membrane-associated cellular components, and despite the fact that most of the antibodies used were raised against the muscle-specific isoforms of microfilament proteins, much has been learned about the architecture of focal contacts by this technique. Another approach comprises the biochemical analysis of crude preparations of focal adhesions obtained by fractionating cultivated cells (by lysis squirting, dry cleavage, or sonication) and identifying the remaining polypeptides (cf Badley et al 1978, Nicol & Nermut 1987, Brands & Feltkamp 1988, Feltkamp et al 1991, Plopper & Ingber 1993). This technique yielded a catalogue of the main elements, but minor or highly soluble components may be lost this way. Both approaches have been supplemented by microinjection of isolated proteins and antibodies, by the construction of genetically manipulated cells, and by extensive analyses on the in vitro properties of individual proteins. Today, we still do not understand every detail of the functional role of the numerous elements involved, but with a combination of the techniques now available, progress should be much faster than a few years ago (cf Gilmore & Burridge 1995).

Several excellent reviews have demonstrated the exponential increase in the number of components and their possible interactions (cf Burridge et al 1988, Critchley et al 1991, Turner & Burridge 1991, Luna & Hitt 1992). Our review should be understood as an update of this work, to provide the reader with the current views on the molecular architecture of focal adhesions. In addition, we also outline some current views on putative regulatory mechanisms.

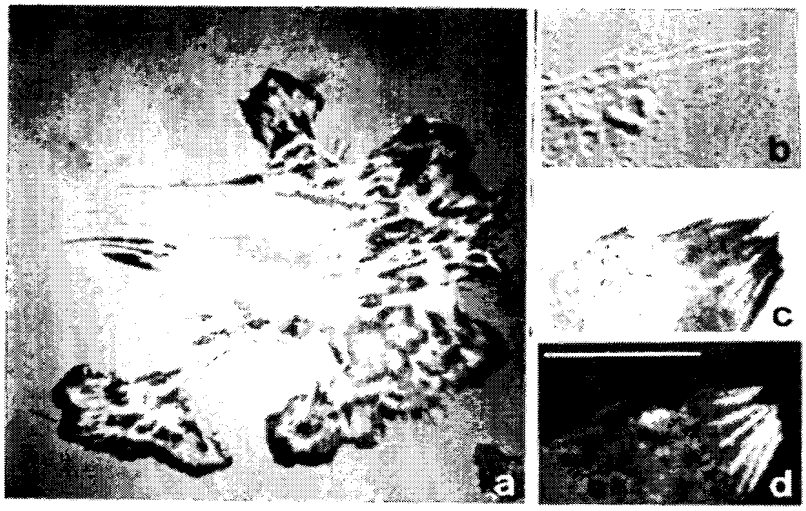

Figure 1 Morphology and shape of focal contacts. (*a*): Primary rabbit fibroblast as seen by reflection interference contrast microscopy. With this technique, the underside of the cell is depicted. Black areas correspond to zones of very close apposition to or direct contact with the bottom of the culture dish. Numerous arrow-shaped black spots are seen at the periphery and in the more proximal regions of several lamellipodia developed by this cell. They correspond to the cell's feet, the focal contacts. Bar: 20 μm. (*b-d*): The edge of a well-spread, immobile chicken fibroblast; (*b*) the dorsal surface as seen by differential interference contrast microscopy; (*c*) the ventral surface as seen in reflection contrast; (*d*) fluorescence image after staining with an antibody against vinculin, a major structural protein of the cytoplasmic face of focal contacts. The focal contacts seen at the cell's underside in (*c*) correspond to the accumulation of vinculin in (*d*). Bar: 10 μm.

STRUCTURAL PROTEINS OF THE CYTOPLASMIC FACE

The cytoskeletal elements forming the cytoplasmic face of focal contacts are part of the microfilament system. Figure 3 presents a schematic view of the various structural and regulatory components and their interactions, as characterized in vitro. Most are localized in various microfilament aggregates. Only two (talin as a structural component and paxillin as a regulatory component) are restricted to cell-matrix adhesions. These two proteins are not found in cell-cell contact regions.

General Building Elements

ACTIN The most abundant protein involved in the construction of the cytoplasmic face of the focal adhesion is actin, and a spot-like concentration of actin at the plasma membrane is the earliest structure that can be defined as a

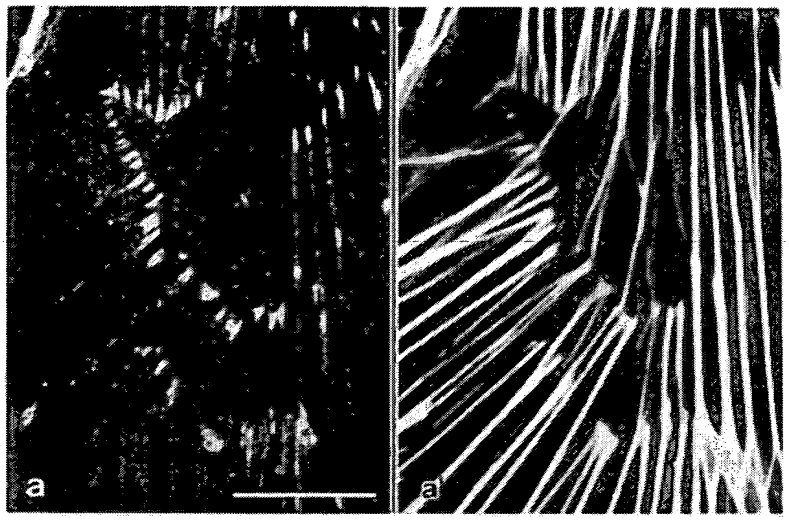

Figure 2 Targeting of vinculin to the termini of microfilament bundles. Mouse 3T3 fibroblasts were extensively extracted with detergents to remove all cellular components except for the cytoskeleton and subsequently incubated with chicken smooth muscle vinculin. The chicken vinculin was monitored by staining with an antibody specific for avian vinculin and rhodamine (a), the microfilament bundles were counterstained with fluorescein-conjugated phalloidin (a'). Every end of a microfilament bundle, indicated by phalloidin staining, is decorated with the chicken vinculin, which demonstrates that the cytoplasmic face of focal contacts, although already containing endogenous vinculin (cf Figure 1), can bind additional vinculin molecules. Bar: 20 μm.

nascent focal contact (De Pasquale & Izzard 1987). In fluorescence micrographs obtained with fluorochrome-labeled phalloidin or anti-actin (Lazarides & Weber 1974, Wulf et al 1979, Wehland et al 1979), as well as in ultrastructural pictures (cf Abercrombie et al 1971, Jockusch et al 1986), the terminal portions of actin filaments are seen tightly bundled near the plasma membrane. This organization is probably achieved by the interaction of the ends of actin filaments with ligand proteins such as vinculin, tensin, and talin (see below). It is generally believed that the filament ends are oriented in parallel, with their positive (fast-growing) ends facing the membrane. This assumption is based on the few examples where the orientation of actin filaments could be directly demonstrated in electron micrographs by decoration with myosin fragment S1 (e.g. in the microvillus; cf Mooseker & Tilney 1975). In accordance with this hypothesis, fluorochrome-labeled actin subunits have been found to add predominantly or exclusively onto the membrane-apposed filament ends (Wang 1984). Actin filaments within the focal adhesion site prob-

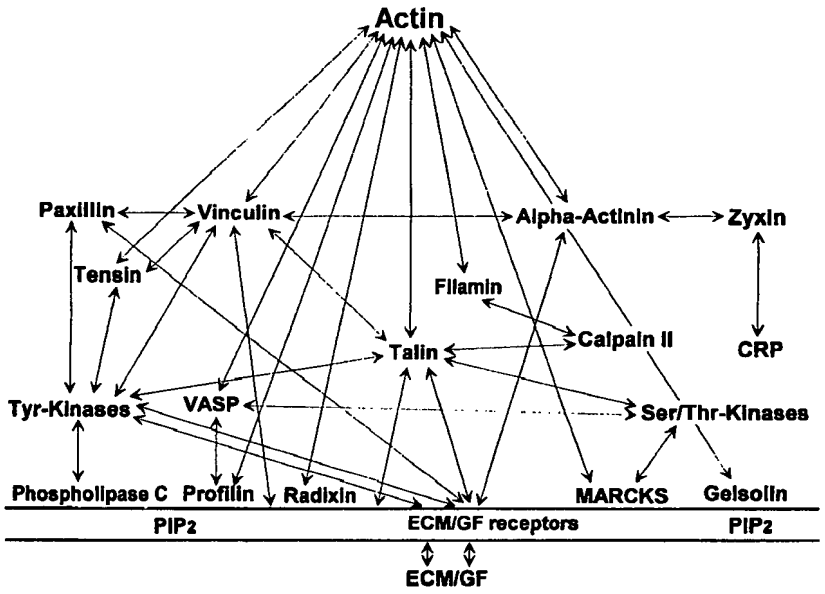

Figure 3 Schematic view of the possible interactions of the main elements of focal adhesions. The arrows illustrate direct interactions, as determined by in vitro assays. The GTP-binding proteins are not included because their precise position within this network is unknown. In the cell, temporally and spatially regulated use of selected bonds seems more likely than the simultaneous establishment of such a network as depicted here. Some principal mechanisms on which such selections may be determined are discussed in the text.

ably contain β and γ actin, and their structure may be deduced from recent articles on muscle actin filaments (Holmes et al 1990). Light microscopic observations on the speed of focal adhesion assembly and disassembly in living, unperturbed cells, as well as in microinjection studies (Wang 1984), suggest that new actin subunits can be rapidly added within seconds or minutes. Whether the ends are capped by an actin-capping protein, which might then also link the actin filaments "end-on" to plasma membrane components, or are freely accessible, is currently under debate (see below).

FILAMIN Filamin, also called actin-binding protein (Wang et al 1975, Hartwig & Stossel 1975), is not exclusively associated with the terminal portions of actin filament bundles. Fluorescence images obtained after microinjection show that this protein, like α-actinin, associates with microfilament bundles in discrete and periodic areas, but homogeneously with the stress fiber termini at focal contacts (Mittal et al 1987). Filamin is a homodimer, with two polypeptide chains arranged in parallel, associated only at their C-terminal ends. Their

N-terminal actin-binding domains are exposed. The result of this arrangement is a long, flexible molecule with the character of a "leaf spring" (Gorlin et al 1990). In accordance with this conformation, filamin is a potent cross-linker and stabilizer of various actin filament aggregates. Depending on the filamin-F-actin ratio, filamin reenforces loose microfilament nets, such as those present in the cell cortex, or tightly packed bundles, as found in stress fibers and focal adhesion sites (Hartwig et al 1980, Hartwig & Stossel 1981).

TENUIN This large (400 kDa) protein has been isolated from cell-cell contact sites, but it was also found as a component of stress fibers, including their terminal portions within the focal adhesion area. Isolated tenuin molecules are approximately 400 nm in length (Tsukita et al 1989); thus they appear to be another rod-like component of the terminal portions of microfilament bundles. However, no further information on sequence, domain structure, ligand binding, or possible function of this molecule is available.

ALPHA ACTININ This protein, a prominent component of myofibrillar Z-bands, dense plaques of smooth muscle, and the dense body-like structures inserted into stress fibers of nonmuscle cells, was found highly concentrated in focal adhesions of many cell types (Lazarides & Burridge 1975, Wehland et al 1979, Sanger et al 1983). It is the prototype of a large family of actin filament cross-linkers composed of two identical polypeptides organized in an antiparallel manner (Figure 4). The evolutionary well-conserved actin-binding sites are exposed at both ends of the rod-like dimeric molecule (for review, see Blanchard et al 1989). The 93–104 kDa large polypeptide chains of α-actinin can be divided into three functional domains: the actin-binding site in the N-terminus, a central region consisting of four α-helical motifs, and a C-terminal domain containing two EF-hands (Noegel et al 1987).

Two genes for α-actinin have been identified that code for skeletal muscle, smooth muscle, and nonmuscle isoforms. Smooth muscle and nonmuscle isoforms are splicing variants of the smooth muscle gene, differing in the region of the first EF-hand (Parr et al 1992, Waites et al 1992). Nonmuscle α-actinin binds Ca^{2+} and is then unable to bind to actin filaments, whereas the muscle isoforms are Ca^{2+} insensitive (Burridge & Feramisco 1981). Interestingly, both forms have been found expressed in nonmuscle cells, and their proportion differs with the microfilament organization of the cell type: fibroblasts with prominent stress fibers and well-developed focal adhesion sites synthesize more of the Ca^{2+}-insensitive form than do transformed and tumor cells. This finding generated the hypothesis that large, long-lasting focal contacts may preferentially contain the isoform not subject to Ca^{2+}-dependent regulation. However, microinjection and transfection studies could not demonstrate a

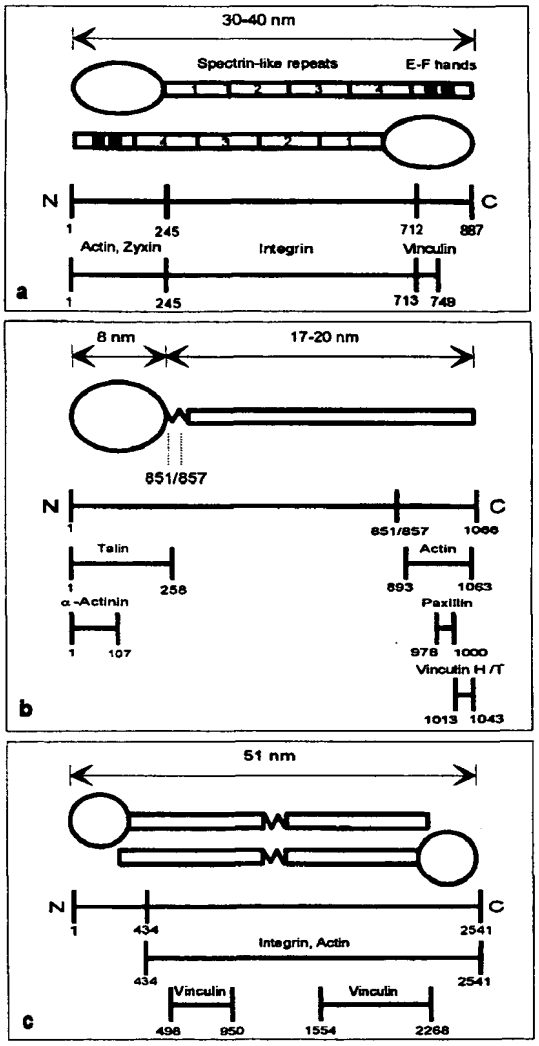

Figure 4 Schematic view of the domain structure of three major structural components of focal contacts: (*a*), alpha actinin; (*b*), vinculin; (*c*), talin. The overall shape of the molecule is given in the top part of each panel. The dimensions were derived from rotary-shadowed preparations. All three molecules consist of a roughly globular head portion and a rod-like tail. In the case of vinculin, these two domains are connected by a proline-rich hinge. A similar hinge has been located in the center of the talin rod. Although α-actinin and talin are dimers, vinculin appears as a monomer. Below the molecules, the ligand-binding sites are depicted in relation to the sequence. The numbers indicate amino acid residues. For details and references, see text.

selective incorporation of the two variants into different cellular sites (Waites et al 1992).

The actin binding-site on α-actinin resides within residues 120–134 (Kuhlmann et al 1992) (Figure 4). Alpha actinins do not bind to G-actin; instead, they contact two subsequent actin protomers along the filament helix (McGough et al 1994). On the actin side, residues 112–125 and 360–372, two sequence stretches located in subdomain 1 of G-actin (Kabsch et al 1990), are involved (Lebart et al 1993, Matsudaira 1994). From its molecular design, as well as from the appearance of actin-α-actinin networks obtained in vitro (Jockusch & Isenberg 1981, Meyer & Aebi 1990), the function of α-actinin might have been deduced predominantly as a cross-linker and spacer between actin filaments, rather than as a protein involved in bundling and packing filament ends as seen in focal adhesion sites. In fact, it is not proven that the α-actinin molecules found concentrated there do indeed cross-link filaments by using both actin-binding sites synchronously, because dimerization of the molecule is apparently not a prerequisite for actin binding (Tokuue et al 1991). In addition to actin, α-actinin has been found to interact with three other proteins of focal adhesion sites: vinculin, β_1 integrin, and zyxin (see below; Figure 4). The binding sites for vinculin and β_1 integrin reside in a 53-kDa fragment of α-actinin that can be obtained by thermolysin cleavage. This fragment is comprised of the internal repeat and the C-terminal portion of the α-actinin molecule (Otey et al 1990, McGregor et al 1994, Kroemker et al 1994). The zyxin-binding site has been located in the N-terminal 27 kDa thermolysin fragment (Crawford et al 1992).

The importance of α-actinin as a structural component of focal contacts is stressed by the observation that alterations in its intracellular level have drastic consequences for cellular adhesion and motility: Overexpression in fibroblasts results in the formation of more stable attachment sites, whereas a general reduction of α-actinin synthesis is associated with an increase in cell motility (Glück et al 1993, Glück & Ben-Ze'ev 1994). Like vinculin, it is also an early response factor: Serum stimulation of fibroblasts leads to increased α-actinin synthesis (Glück et al 1992).

VINCULIN This protein is highly concentrated in focal contacts (Geiger 1979, Burridge & Feramisco 1980, Schliwa & Potter 1986), smooth muscle dense plaques (Small 1985), striated muscle Z-lines (Pardo et al 1983), and platelet attachment plaques (Nachmias & Golla 1991; see also Otto 1990, for review). Vinculin elutes as a single 116-kD polypeptide chain from columns in physiological buffers. From nematodes to humans, there is only one gene identified for vinculin (Weller et al 1990, Barstead & Waterston 1991). In mammalian fibroblasts, three isoforms have been identified that may be the result of different phosphorylation states. Focal adhesions appear to consist of primarily

the most acidic form of vinculin (Geiger 1982). Smooth and cardiac muscles synthesize two splice forms, vinculin and metavinculin. Metavinculin, which was also identified in platelets (Turner & Burridge, 1989), contains an additional sequence stretch as an insert (Feramisco et al 1982, Gimona et al 1988, Belkin et al 1988).

In electron micrographs of mica-adsorbed, metal-shadowed preparations, chicken gizzard vinculin appears as a molecule composed of two morphologically distinct regions: an approximately globular head and a rod-like tail (Milam 1985, Molony & Burridge 1985). This asymmetry is also displayed under physiological conditions (Eimer et al 1993). Sequence analyses have proposed that the two structural domains are linked by a proline-rich hinge (Price et al 1987, 1989; Coutu & Craig 1988). The functional domains have been analyzed by studying the intact protein and its proteolytic fragments (Jockusch & Isenberg 1981, Otto 1983, Wilkins et al 1983, Burridge & Mangeat 1984, Wachsstock et al 1987, Belkin & Koteliansky 1987, Groesch & Otto 1990, Turner et al 1990, Pavalko & Burridge 1991, Menkel et al 1994, Kroemker et al 1994) and partial sequences expressed as recombinant fusion proteins (Jones et al 1989, Gilmore et al 1992, McGregor et al 1994, Menkel et al 1994). These studies revealed that vinculin binds to actin, α-actinin, talin, and paxillin and can also form self-aggregates (Figure 4). The first 120 N-terminal residues, located within the head portion, are required for the binding of talin (Burridge & Mangeat 1984, Jones et al 1989, Gilmore et al 1992) and α-actinin (Kroemker et al 1994), but their topographical location within the globular head is not known. The binding sites for paxillin and filamentous actin reside within the rod-like tail portion (Wood et al 1994, Menkel et al 1994, Johnson & Craig 1995). The existence of an F-actin binding site has been the subject of controversy (cf Jockusch & Isenberg 1981, 1982; Wilkins & Lin 1986, Otto 1986, 1990; Ruhnau & Wegner 1988, Westmeyer et al 1990) but was recently unequivocally demonstrated by quantitative sedimentation assays, using recombinant vinculin (Menkel et al 1994, Johnson & Craig 1995). In addition, the vinculin tail fragment has been shown to bind to acidic phospholipids (Ito et al 1983, Niggli et al 1986, Isenberg 1991) and to phosphatidylinositol-4,5-bisphosphate, PIP_2 (Fukami et al 1994). Intramolecular head-to-tail associations were described by cross-linking and ligand-binding studies (Kroemker et al 1994, Johnson & Craig 1994, 1995). These data, together with the finding that isolated vinculin heads and tails bind their respective ligands much more strongly than the intact molecule, suggest the existence of folded vinculin molecules that have to open up for ligand interaction. Recently, we were able to visualize such folded molecules in negatively stained electron micrographs (Figure 5).

Modulation of the vinculin expression level has serious consequences on cellular attachment and motility, quite similarly to what was found for α-act-

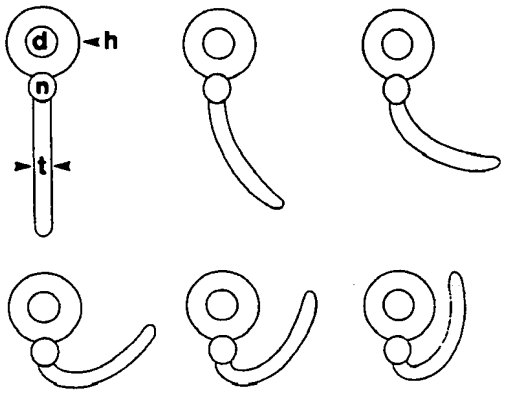

Figure 5 Gallery of vinculin molecules represented by schematic drawings taken from electron micrographs of negatively stained preparations. The main features are a head domain (h), which contains a protein-deficient center (d), a neck region (n), probably corresponding to the proline-rich hinge indicated in Figure 4*b*, and a rod-like tail (t). Although the entire tail appears moderately flexible, the neck confers a region of extreme flexibility to the molecule. As a consequence, vinculin can adopt various conformations ranging from an extended, open form to a closed jack-knife configuration. In the latter, head-to-tail association is established and ligand binding is impaired (see text).

inin: A decrease of vinculin levels results in an increase of motility, impaired cell attachment, and unstable lamellipodia (Rodriguez Fernandez et al 1993, Varnum-Finney & Reichardt 1994). Conversely, stimulation of cell proliferation by serum induces vinculin synthesis as an early response (Ungar et al 1986).

Actin Filament-Capping Proteins

The notion that the positive, fast-growing ends of actin filaments in focal adhesion sites face the plasma membrane has for a number of years stimulated discussions on whether these ends are free or capped by specific proteins. Recent studies have presented several good candidates for such putative capping proteins. They can apparently also interact with integral membrane proteins, which classifies them as potential interprotein links.

RADIXIN This 82-kD protein is a member of the so-called band 4.1 protein family, which comprises, in addition to radixin, its close relatives ezrin and moesin, band 4.1 protein (first identified in erythrocytes as the glycophorin anchor of the submembraneous cytoskeleton), and talin (Rees et al 1990, Lankes & Furthmayr 1991, Funayama et al 1991). In their N-terminal half, these proteins contain a domain with high sequence similarity. Ezrin, moesin, and radixin are closely related but are products of different genes (Funayama

et al 1991). Because of their sequence similarity (approximately 75% identity), discrimination of their cellular localization with specific antibodies was difficult. Recent evidence indicates that radixin, which was first described as a component of microfilament bundles in cleavage furrows and cell-to-cell contacts (Tsukita et al 1989, Sato et al 1991), is also a component of focal adhesions, in contrast to ezrin and moesin, which seem to be excluded from this location (Sato et al 1992, Franck et al 1993). Radixin and ezrin contain proline clusters (Funayama et al 1991), and all three proteins interact with the integral membrane protein CD44, as has been shown by immunoprecipitation (Tsukita et al 1994). Radixin also contains an actin-binding domain, probably located in its C-terminal part. Radixin binding to actin interferes with the addition of actin subunits at the positive end of the F-actin filament. Thus it may serve as a linker between the ends of actin filaments and an integral plasma membrane component (Tsukita et al 1989, Bretscher 1993).

TENSIN/INSERTIN Tensin, a protein composed of two 200-kD polypeptide chains, has been described as a component of different microfilament-membrane contacts, including the Z-lines of myofibrils (Wilkins et al 1986, Bockholt et al 1992, Lo et al 1994b). It is highly enriched in the distal portions of stress fibers. Its molecular configuration and functional domain structure have been extensively studied. Tensin contains three distinct, actin-binding sites per chain, enabling this protein to cap as well as cross-link actin filaments (Lo et al 1994 a,b). The capping activity is weak and confined to a region identical in sequence with a protein called insertin (Weigt et al 1992). In the presence of insertin, actin filaments appear to be capped only on one of the two terminal subunits at the positive end of the filament, thus allowing for slow growth at this end (Ruhnau et al 1989). At present, the relationship between insertin and tensin is not clear: Insertin might be a proteolytic fragment, a splice variant, or the product of a related gene (A Wegner, personal communication). In addition to three actin-binding sites, tensin apparently possesses a vinculin-binding (Lo et al 1994b) and a Src homology 2 (SH2) domain (Davis et al 1991; see below). Tensin can also self-associate via its C-terminus. The characterization of these various functional domains has led to the speculation that tensin forms U-shaped molecules, embracing the ends of actin filaments and interacting with membrane-located SH2-binding proteins (Lo et al 1994 a,b). Tensin itself is phosphorylated on tyrosine after the induction of focal contact formation by extracellular ligands (Bockholt & Burridge 1993; see below). Therefore, controlled, slow filament growth, bundling, and stabilization of the newly formed filament ends would be conferred to the focal adhesion site by tensin, a multifunctional protein combining structural properties with those of a regulatory component.

TALIN Thus far talin (Burridge & Connell 1983 a,b) is the only structural component of cell-matrix contact sites specifically excluded from cell-cell contacts (Geiger et al 1985, Drenckhahn et al 1988). It is concentrated in focal contacts, myotendinous junctions, dense plaques of smooth muscle, and the adhesion plaques of activated platelets, but is also found in the cortical microfilament web of ruffling membranes (Burridge & Connell 1983a,b; Geiger et al 1985, Tidball et al 1986, Beckerle et al 1987, 1989; Beckerle & Yeh 1990). The protein, a homodimer of 2 × 270 kDa (Rees et al 1990), with two polypeptides arranged in antiparallel orientation, forms an approximately 50-nm long rod with two globular ends, possibly comprising the N-terminal domains (Molony et al 1987, Goldmann et al 1994; Figure 4). Limited proteolysis with calpain II or thrombin has been employed to separate a 47-kD N-terminal from a 200-kD C-terminal fragment (Fox et al 1985, Beckerle et al 1987, Niggli et al 1994). After microinjection, both fragments can independently target to focal contacts (Nuckolls et al 1990). The N-terminal portion, which has sequence similarity to members of the band 4.1 protein family (Rees et al 1990), can directly interact with phospholipids and membranes (Niggli et al 1994), whereas the large C-terminal fragment interacts with β_1 integrin (Horwitz et al 1986), actin (Muguruma et al 1990, Kaufmann et al 1991, Niggli et al 1994), and vinculin (Burridge & Mangeat 1984, Gilmore et al 1993; Figure 4). In analogy to the question of vinculin-actin interaction, the direct binding of talin to actin is also controversial (cf Beckerle & Yeh 1990, Isenberg & Goldmann 1992), but recent in vitro data suggest that talin can nucleate, cap, and cross-link actin filaments (Muguruma et al 1990, 1992; Goldmann & Isenberg 1991, Kaufmann et al 1991, Goldmann et al 1992). Studies with fusion proteins containing various talin segments revealed two vinculin-binding regions that are not adjacent in the sequence. Talin is phosphorylated by serine/threonine kinase, as well as by tyrosine kinase (Bertagnolli et al 1993). In platelets, talin relocates during activation from a cytoplasmic, diffuse distribution to newly formed adhesion sites (Beckerle et al 1989), concomitant with an increase in its phosphorylation state (Bertagnolli et al 1993).

REGULATORY PROTEINS OF THE CYTOPLASMIC FACE

In contrast to the putative structural components described above, regulatory proteins are thought to modulate the microfilaments within the focal adhesion site. They are present in much smaller amounts than the structural components and are probably primarily involved in the dynamic aspects of focal adhesions.

Actin Filament Regulators

As elsewhere in the cell, actin polymerization at the plasma membrane has to be finely regulated. Two candidate proteins involved in this process are profilin

and gelsolin. Both bind to monomeric actin and to phospholipid components. In addition, the myristoylated alanine-rich C-kinase substrate (MARCKS) is considered as a putative regulator of the arrangement of actin filaments close to the membrane.

PROFILIN This small (12–17 kDa) protein was first characterized as a G-actin-binding protein from bovine tissue (Carlsson et al 1977). More recently, profilins have been found in all eukaryotic cells and tissues, although in different amounts (Buss & Jockusch 1989). Several profilin genes have been identified, and different isoforms can be expressed in the same tissue (Honoré et al 1993; see Haarer & Brown 1990, Machesky & Pollard 1993 for additional references). Profilin has been associated with microfilaments in highly dynamic areas of the cell, such as the leading lamella and the tips of nascent stress fibers of fibroblasts (Buss et al 1992). Although of limited sequence similarity, all profilins analyzed so far display a strikingly similar structure, as seen by NMR and X-ray studies: A core of antiparallel β-pleated sheets is flanked by α-helical regions, with the N- and C-termini located at the same side of the molecule (Vinson et al 1993, Metzler et al 1993, Schutt et al 1993, Fedorov et al 1994, Pollard et al 1994). Isolated profilins bind to actin, the lipid phosphoinositide PIP_2, (Lassing & Lindberg 1985), and to poly-L-proline (Tanaka & Shibata 1985, Lindberg et al 1988), and it is believed that profilins use all three relevant binding sites in regulating actin polymerization. Candidate ligands for the poly-L-proline binding motif are discussed below. The interaction of profilin with actin is much more complex than previously believed. In addition to sequestering G-actin, which lowers the concentration of free subunits and thus may delay polymerization and induce depolymerization of actin filaments, profilin stimulates the ATP/ADP exchange on G-actin. Because ATP is abundant in cells, this process favors filament polymerization. Moreover, the profilin-actin complex can directly interact with the fast growing (membrane-apposed) end of the actin filament (see Goldschmidt-Clermont et al 1992, Pantaloni & Carlier 1993, Theriot & Mitchison 1993, Sohn & Goldschmidt-Clermont 1994 for references). Binding to monomeric actin and PIP_2 is mutually exclusive, which has led to the view that profilins may be at the crossroads between signal transduction and the membrane-attached microfilament system (Aderem 1992, Machesky & Pollard 1993, Sohn & Goldschmidt-Clermont 1994; Figure 6, see also Figure 7). Topographical analyses suggest that the actin-binding domain (Vandekerckhove et al 1989, Vinson et al 1993, Schutt et al 1993) is adjacent to a hydrophobic patch comprising the PIP_2-binding site (Schutt et al 1993, Vinson et al 1993, Fedorov et al 1994). Binding to poly-L-proline is independent of actin- and PIP_2-binding, and requires several highly conserved aromatic amino acid residues and 6–10 proline residues in tandem on the ligand side (Björkegren et al 1993, Metzler et al 1993, Archer

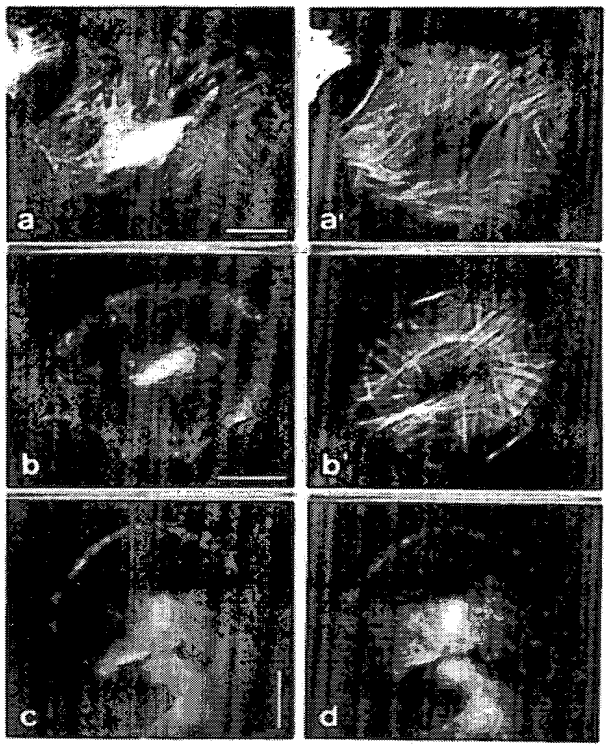

Figure 6 Immunofluorescence images of spreading rat fibroblasts that reveal the codistribution of profilin and VASP by specific antibodies: (*a,a'*) double fluorescence of VASP distribution seen by antibody/rhodamine staining (Courtesy of M Reinhard & U Walker) and actin by fluorescein-conjugated phalloidin; (*b,b'*) double fluorescence of profilin revealed by antibody/rhodamine staining and actin by fluor- escein-conjugated phalloidin; (*c,d*) double fluorescence of VASP and profilin. Both proteins are codistributed in the small, nascent focal contacts at the periphery of spreading cells. Bars: 30 μm.

et al 1994). Cellular candidates exploiting the poly-L-proline-binding motif are discussed below.

GELSOLIN Similarly to profilin, gelsolin (Yin 1987) is associated primarily with specific types of focal adhesion sites. It has been identified as a component of podosomes in transformed fibroblasts (Wang et al 1984). Gelsolins are members of a large superfamily. The polypeptide chain of the most frequent form occurring in animal cells is approximately 80 kDa in size and contains multiple actin-binding sites (Janmey 1993). Gelsolins cut (sever) actin fila-

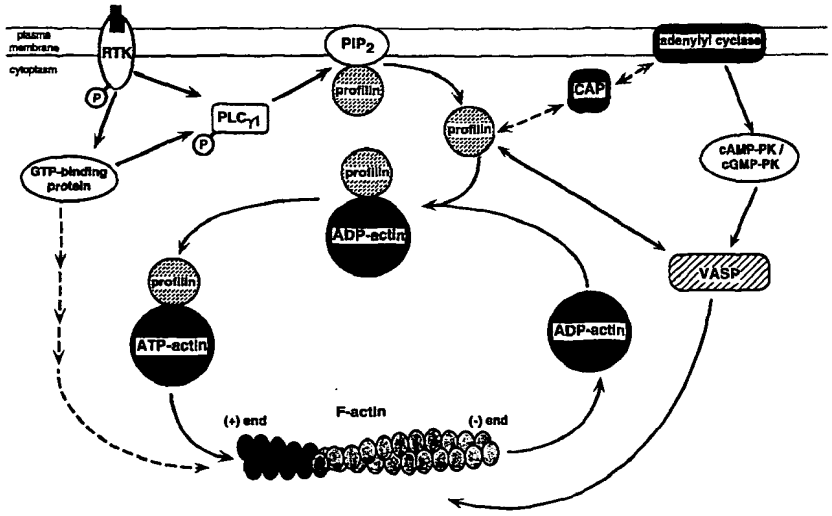

Figure 7 Schematic view of profilin and VASP as mediators between the microfilament system and signal transduction. RTK, receptor tyrosine kinase; PLCγ1, phospholipase Cγ1. Dotted arrows point to putative interactions that may involve further components. VASP and profilin interact with actin and with each other, linking two different signal transduction pathways to the actin filament system. For further explanation, see text.

ments in a Ca^{2+}-dependent manner, cap the ends of filaments, and nucleate filament assembly (see Vandekerckhove 1990, Hartwig & Kwiatkowski 1991 for references). The complex of the first (N-terminal) actin-binding domain with G-actin was crystallized, and the structural data show that this domain superficially resembles profilin. It also comprises a core of antiparallel β-pleated sheets flanked by α-helical regions. Like profilin, it also contacts subdomains 1 and 3 of actin, but the contact involves parts of the molecule that are not equivalent to the profilin-actin interface and is much tighter (McLaughlin et al 1993), which explains the higher affinity of gelsolin for actin, as compared with profilin (Rozycki et al 1994, Ampe & Vandekerckhove 1994). Thus its capping activity at the positive end of the actin filament is much more powerful. Gelsolin has been found to bind to PIP_2 (Janmey & Stossel 1989), and a corresponding binding site has been identified in domains 1 and 2 of gelsolin (Yu et al 1992, Janmey et al 1992). These sites show conserved sequence motifs. As described for profilin, PIP_2-binding to gelsolin is incompatible with actin-binding (Janmey et al 1987), which suggests a modulatory role of gelsolins in signal-induced microfilament reorganization at the plasma membrane.

MARCKS The myristoylated alanine-rich C-kinase substrate, a 68–87 kDa phosphoprotein (Stumpo et al 1989), may also participate in regulating the organization of actin filaments at the plasma membrane. It contains an F-actin-binding domain (Janmey et al 1993) and colocalizes with actin, vinculin, talin, and protein kinase C (see below) in small punctate spots at the adherent surface of filopodia, which are another subset of focal adhesive contacts (Rosen et al 1990). In addition, it binds to Ca^{2+}/calmodulin. This activity, as well as its association with the plasma membrane, is apparently regulated by serine phosphorylation: When phosphorylated by protein kinase C, the protein redistributes to the cell interior, and dephosphorylation targets it back to the plasma membrane, where it binds to Ca^{2+}/calmodulin and cross-links actin filaments (Aderem 1992, Janmey et al 1993).

Proline Motif Proteins

The ability of profilins to bind to poly-L-proline has prompted the search for natural ligands containing proline clusters. Recently, the first of such putative profilin partners was identified as the vasodilator-stimulated phosphoprotein (VASP). This 46/50-kD phosphoprotein was originally characterized in platelets as a substrate of both cGMP- and cAMP-stimulated protein kinases, enzymes that are essential for the control of platelet activation (Halbrügge et al 1990). VASP is not confined to platelets, however, but has been found in many cells and tissues (Reinhard et al 1992, Halbrügge et al 1992, Haffner et al 1995, Reinhard et al 1995). It has been identified as a component of focal adhesion sites by immunofluorescence and as an F-actin-binding protein in sedimentation assays (Reinhard et al 1992). In spreading cells, it colocalizes with profilin in nascent focal contacts (Figure 6). Cloning and sequencing of the human and canine proteins (Haffner et al 1995) have revealed a proline-rich core region, with a $G(P)_5$ motif present as a single copy and a threefold tandem repeat. Structural predictions based on this sequence suggest that these proline runs are surface-exposed on a rod-like domain and may thus be available for protein-protein interactions. Experiments performed with animal and plant proteins indicate that mammalian VASP binds directly to a wide variety of profilins, via the $G(P)_5$ motif (Reinhard et al 1995). It is conceivable that VASP is a representative of a larger group of proteins that interact with profilin by proline motifs, but thus far the $G(P)_5$ motif is unique to VASP (Haffner et al 1995, Reinhard et al 1995). These data suggest that profilin's role in actin filament assembly at the focal contact site may not only be controlled by the PIP_2 signal transduction pathway, but also with VASP as a mediator, by cAMP/cGMP-dependent pathways (Figure 7). This regulation might even operate while profilin acts on actin, because in contrast to PIP_2 binding, VASP binding to profilin is compatible with actin binding. Transfection experiments with truncated mutants have shown that VASP also contains a focal contact

targeting sequence in its C-terminal domain, independent of the $G(P)_5$ motif (Haffner et al 1995).

Further candidate ligands using proline clusters for binding to profilins are radixin (Funayama et al 1991), zyxin (see below; Sadler et al 1992), and the CAP-like proteins. The CAP (adenylate cyclase-associated) protein identified in yeast (Field et al 1990) and its relatives, the CAP-like proteins in hydra (Fenger et al 1994), human (Matviw et al 1992), and pig platelets (Gieselmann & Mann 1992), all have conspicuous proline-rich motifs. The pig protein also binds to actin (Gieselmann & Mann 1992). Genetic studies in yeast have provided good evidence that a fine balance between profilin and CAP is important for microfilament-associated functions (Vojtek et al 1991).

LIM Proteins

LIM domains are rather long (47–59 amino acid residues; Freyd et al 1990), cysteine-rich consensus sequences that bind to and are structurally stabilized by two atoms of zinc per domain (Kosa et al 1994). LIM domain-containing proteins are common among transcription factors and proto-oncogenes, but the discovery that they also occur among cytoskeletal components is rather novel (for review, see Sánchez-Garciá & Rabbitts 1994). The analysis of recombinant LIM proteins and of deletion mutants suggests that the LIM domains can mediate protein-protein interactions (Schmeichel & Beckerle 1994). Thus far three LIM proteins, all of low cellular abundance, have been described as being associated with the cytoplasmic face of focal adhesions.

ZYXIN This protein, approximate molecular weight of 82 kDa, has been localized in focal adhesions, smooth muscle dense plaques, and cell-cell contacts of retina epithelial cells (Beckerle 1986, Crawford & Beckerle 1991). It is a phosphoprotein that contains binding sites for α-actinin and another LIM protein, CRP (see below). A binding site for the 27-kDa N-terminal fragment of α-actinin has been located to the N-terminal two thirds of the molecule (Schmeichel & Beckerle 1994), which also harbors proline-rich sequence motifs (Sadler et al 1992). The C-terminal third contains three LIM domains in tandem, a feature not found in any other LIM protein (Sadler et al 1992).

CRP This small (20 kDa) globular protein has been isolated from smooth muscle where its expression is apparently developmentally regulated (Crawford et al 1994). The protein contains two LIM domains. Zyxin and CRP associate via their LIM motifs, and the first of the three domains in zyxin is sufficient to mediate this interaction (Schmeichel & Beckerle 1994).

PAXILLIN Like talin, this 68-kD protein is an adherens junction component not found in cell-cell contact sites (Turner et al 1990). But paxillin is much

less abundant than talin and probably plays a regulatory rather than structural role. Paxillin has attracted the attention of many groups because it appears to interact with many cytoskeletal components and may play a functional role in vivo. The C-terminal third of the molecule contains one LIM and three LIM-like domains (Turner & Miller 1994). The N-terminal half interacts with the vinculin rod-like tail and the focal adhesion kinase (FAK) (see below). The N-terminal domain is subject to tyrosine phosphorylation by FAK and several other kinases (Birge et al 1993, Weng et al 1993, Sabe et al 1994, Turner 1994), and paxillin may subsequently interact with proteins containing SH2 domains. A proline-rich consensus recognition motif for SH3 domains is also located in the N-terminal region (Turner & Miller 1994).

Proteases

Proteolytic cleavage of the cytoskeletal elements at the focal contact has also been discussed as a possible regulatory mechanism. Calpain II, a Ca^{2+}-stimulated thiol protease, has been characterized as a component of focal adhesions in fibroblasts (Beckerle et al 1987, Burridge et al 1988) and is associated with the plasma membrane during integrin-dependent platelet activation (see below; Fox et al 1993). Both platelet talin and filamin have been identified as calpain II substrates (Collier & Wang 1982, O'Halloran et al 1985, Fox et al 1985).

Phospholipases

The involvement of the γ1 isoform of phospholipase C in regulating the focal adhesion sites and cellular attachment is indicated in many studies. This membrane-associated enzyme is a key factor in generating important signaling molecules from PIP_2. The important pathway in the context of this review involves EGF- and PDGF-receptor activation, which leads to phospholipase Cγ1 phosphorylation and its subsequent activation. The activation results in conversion of PIP_2 to diacylglycerol and inositol 1,4,5-trisphosphate (IP_3), even when the latter is still complexed to profilin (Goldschmidt-Clermont et al 1990, Aderem 1992, Heldman & Goldschmidt-Clermont 1993). The consequences for actin polymerization, inositol phospholipid turnover, and the relation to the GTP-binding proteins are discussed below.

Protein Kinases

This class of proteins has attracted attention by scientists interested in focal adhesions for two reasons: First, several kinases have been localized in focal adhesions where they may be engaged in modulating the interaction of microfilament components by phosphorylation. Second, the findings that many receptor tyrosine kinases directly associate with F-actin has greatly stimulated the concept of the focal adhesion as a structure with dual functions: a site for

cell anchoring, as well as for mediating signal transduction. Selected examples of focal adhesion-associated kinases are given below.

SERINE/THREONINE KINASES Several kinases of this type have been shown to phosphorylate focal adhesion components in vitro and in living cells. This has been described in detail for cGMP- and cAMP-dependent protein kinases (PK) and their substrate VASP (Reinhard et al 1992, Walter et al 1993, Eigenthaler & Walter 1994). In blood platelets, VASP is sequentially phosphorylated at two serine and one threonine residues by cGMP- and cAMP-PK, respectively (Butt et al 1994). This is an important step in the inhibitory pathway controlling the reorganization of the platelet microfilament system during activation (Walter et al 1993). VASP can also be phosphorylated by cGMP- and cAMP-PK in fibroblasts, without obvious consequences for the morphology of focal adhesion sites (Reinhard et al 1992). Thus the role of VASP phosphorylation at these areas remains to be elucidated (Butt et al 1994).

Protein kinase C has been localized in the small punctate adhesion sites of filopodia in macrophages, where it codistributes with MARCKS (Rosen et al 1990, Aderem 1992) and supposedly regulates the function of this protein in linking actin filaments to the plasma membrane. Vinculin and talin have also been identified as substrates for this membrane-associated kinase (Beckerle 1990). The α and δ isoforms have been found enriched in nascent focal adhesions (Jaken et al 1989, Woods & Couchman 1992, Barry & Critchley 1994).

TYROSINE KINASES Tyrosine kinases of the Src family, a large group of membrane-associated enzymes, have been described as focal contact components. Src kinases are characterized by the Src homology regions, SH2 and SH3, which are involved in protein-protein interactions. SH2 domains recognize individual phosphotyrosine residues on ligand proteins, whereas SH3 domains bind to short, proline-rich consensus sequences (see Marengere & Pawson 1994, for references). Both domains may also play an important role in protein-protein interactions at the focal adhesion site. V-Src, a viral enzyme encoded by retroviruses, has been described as being associated with the podosomes of Rous sarcoma virus (RSV)-transformed chicken fibroblasts (Rohrschneider 1980). Its cellular counterpart c-Src shows no obvious association with the focal contacts, even in cells overexpressing it (David-Pfeuty & Nouvian-Dooghe 1990). However, inhibiting the phosphorylation of a single tyrosine (Y527) in c-Src, which is absent in v-Src, results in a striking redistribution of c-Src and targeting to the focal adhesion site (Kaplan et al 1994). Phosphorylation of Y527 downregulates the kinase activity (Courtneidge 1985) and the SH2 domain accessibility (MacAuley & Cooper 1989). The data available until now are consistent with the view that targeting to focal contacts

is independent of the kinase activity (Kaplan et al 1994). c-Src, v-Src, and several related tyrosine kinases (Fyn, Csk) interact with FAK (see below). The interaction of v-Src with FAK is mediated through the SH2 domain of v-Src (Xing et al 1994, Schaller et al 1994), whereas both SH2 and SH3 domains may participate in paxillin binding (Birge et al 1993, Weng et al 1993, Sabe et al 1994, Turner & Miller 1994). Several other focal adhesion proteins such as talin and vinculin have been identified in vitro as substrates for Src kinases (see Hunter & Simon 1993).

FAK, a tyrosine kinase of 125 kDaa, is particularly interesting with respect to focal adhesions, where it is highly enriched in many cell types (Schaller et al 1992). FAK is also found in the microfilament-membrane attachment sites of activated platelets (Choi et al 1993). In contrast to the Src kinases, this enzyme is a peripheral membrane protein that has drawn particular attention because it is activated by autophosphorylation when the plasma membrane contacts extracellular matrix components and also by stimulation of the rho-activated signaling pathway (see below; Schaller & Parsons 1994, Barry & Critchley 1994). It is suspected to act as a master tyrosine kinase for many cytoskeletal elements of the focal contact (Burridge et al 1992, Hanks et al 1992, Guan & Shalloway 1992, Lipfert et al 1992, Chrzanowska-Wodnicka & Burridge 1995), as well as for Src kinases (Cobb et al 1994).

FAK is highly conserved, with a catalytic center flanked by large N- and C-terminal domains. Several studies have shown that the N-terminal sequence binds to integrins (see below), whereas the C-terminal portion interacts with paxillin and mediates the targeting to focal contacts (Schaller & Parsons 1994). Autophosphorylation on tyrosine (in particular, Y397) is induced by integrins, several growth factors, neuropeptides, and lysophosphatidic acid (LPA), and triggers the interaction with Src kinases. This event is correlated with the phosphorylation of the FAK ligand paxillin (Schaller & Parsons 1994, Parsons et al 1994, and references therein). However, neither integrin- nor paxillin-binding is apparently solely responsible for directing FAK to focal adhesions: The C-terminal domain, which is autonomously expressed in certain cell types (Schaller et al 1993), contains such a focal adhesion targeting sequence that largely overlaps, but does not superimpose, the paxillin-binding region (Schaller & Parsons 1994). FAK is not only activated early during focal contact formation but, together with paxillin, is also recruited to the nascent structures (Barry & Critchley 1994).

There is increasing evidence that growth factors like EGF and PDGF substantially modulate focal adhesion sites, a process mediated by the kinase activity of their receptors. Both receptors are monomeric transmembrane proteins, with a cytoplasmic catalytic domain that is activated by ligand binding and autophosphorylation (Hunter & Cooper 1985, Honegger et al 1989, 1990; Ullrich & Schlessinger 1990, Koch et al 1991) and can be modulated by

effector molecules binding to sequences located either adjacent to or within the catalytic sequence. With respect to their involvement in focal adhesion regulation, four of their multiple activities should be mentioned: (*a*) EGF and PDGF receptors both phosphorylate phospholipase Cγ1 (Meisenhelder et al 1989, Morrison et al 1990), a prerequisite for the dissociation of the profilin:PIP_2 complexes (see Figure 7) and possibly also the gelsolin:PIP_2 complexes (Goldschmidt-Clermont et al 1990, Heldman & Goldschmidt-Clermont 1993). (*b*) The EGF receptor contains an F-actin binding domain (den Hartigh et al 1992). (*c*) The PDGF receptor kinase phosphorylates talin, FAK, and paxillin (Tidball & Spencer 1993, Rankin & Rozengurt 1994) as well as several Src kinases (Kypta et al 1990). (*d*) PDGF and EGF have been found to induce focal adhesion formation, probably because their receptors, when activated by ligand binding, stimulate a GTP-binding protein of the ras family (see below).

GTP-Binding Proteins

The relationship between GTP-binding proteins and the actin cytoskeleton has recently been reviewed (Hall 1994). Therefore, we only briefly summarize the evidence for the involvement of these proteins in focal contacts.

Two of the small, monomeric GTP-binding proteins (ras-related GTPases) have been identified as potent regulators of focal adhesion sites. In serum-starved, quiescent Swiss 3T3 fibroblasts, microinjected rhoA induces the formation of stress fibers and focal contacts, and the same effect is seen after addition of LPA or the neuropeptide bombesin (Paterson et al 1990, Ridley & Hall 1992). Rac, a related GTP-binding protein, induces primarily membrane ruffling and, after a slight delay, also induces the formation of stress fibers and focal contacts. Experimental evidence suggests that LPA, bombesin, and rac induce these processes, as part of one or several signal transduction pathways, by raising the cellular level of activated rhoA (Hall 1992, 1994; Ridley & Hall 1992, Ridley et al 1992, Ridley 1994). In addition, rhoA may interact with receptor kinases independently of rac. It is apparently also required for maintenance of focal adhesion sites: When rhoA is inactivated by C3 transferase, an enzyme of *Clostridium botulinum*, stress fibers, and focal adhesions are destroyed (Paterson et al 1990, Ridley & Hall 1992). In activated mast cells, rhoA has been found responsible for de novo actin polymerization (Norman et al 1994). Thus one could deduce from these data that the rho-dependence of focal adhesion site formation and maintenance is also associated with net actin polymerization. However, the molecular links between rho and actin filaments or focal adhesions are not known.

The large, heterotrimeric GTPases also participate in focal contact regulation. In several cell types, the γ5 subunit has been localized within cell-matrix attachment sites (Hansen et al 1994). Because several receptor kinases and adenylate cyclases are now known to be regulated by heterotrimeric GTPases,

we expect that more information regarding the role of these enzymes on focal contact formation will be available soon.

THE TRANSMEMBRANE CONNECTORS

Several families of transmembrane proteins participate in linking actin filaments directly or indirectly to the plasma membrane (see above). The most important class of proteins in this context are the integrins that link the cytoskeletal elements at the cytoplasmic face with extracellular matrix (ECM) components.

Integrins

These heterodimeric transmembrane proteins are composed of α and β subunits, and families of related molecules have been described. Both subunits are composed of a large extracellular glycosylated region responsible for heterodimeric association, a single membrane-spanning domain, and a cytoplasmic domain, which in most cases is only 40–60-amino acid residues in length (Albelda & Buck 1990, Hynes 1992, Sastry & Horwitz 1993). Different α and β chains can form a complex, exposing specific ligand-binding domains for extracellular matrix proteins and for different cytoskeletal elements. Thus different subsets of integrins mediate adhesion to the ECM or to surface proteins on neighboring cells in cell-cell contacts.

Integrins display a functional redundancy: The same integrin heterodimer can recognize several ECM proteins, and a particular ECM ligand may be recognized by more than one integrin (Hynes 1992, Damsky & Werb 1992). For example, several integrins composed of different α and β chains recognize the sequence RGD, which is present in fibronectin, vitronectin, laminin, and other extracellular adhesion proteins. In addition, in several major ECM proteins such as fibronectin and laminin, other motifs participate in integrin-binding (Haas & Plow 1994, Kühn & Eble 1994). In focal adhesion sites, several integrins colocalize with actin, vinculin, talin, and α-actinin. In vitro, a direct interaction of the central region of the cytoplasmic domain of β_1 subunit with α-actinin, talin, and FAK has been described (Horwitz et al 1986, Otey et al 1990, 1993; Schaller & Parsons 1994). Further evidence for the importance of the cytoplasmic domain of the β_1 subunit comes from data showing that mutants or isoforms altered in this region fail to associate with focal contacts (Reszka et al 1992, Balzac et al 1993) and may even inhibit focal adhesion and cell spreading (Balzac et al 1994). The $\alpha_{IIb}\beta_3$ integrin, expressed in high copy number on thrombocytes, plays an essential role as the fibrinogen receptor in platelet aggregation (Ginsberg et al 1992, Shattil 1993). Association of this integrin with the microfilament system during platelet activation has been described (Bertagnolli & Beckerle 1993). A large body of evidence suggests

that in platelets and fibroblasts, integrins must be activated to serve as structural links between the ECM and microfilament proteins at the cytoplasmic face of the focal contact site (e.g. Ginsberg et al 1992, Damsky & Werb 1992, Schaller & Parsons 1994, Williams et al 1994). This activation probably comprises conformational changes in the heterodimer that, on either side of the plasma membrane, may be induced by the ligands themselves and modulated by processes involving other transmembrane receptors such as receptor kinases.

THE EXTRACELLULAR LIGANDS

Small extracellular ligands such as growth factors and hormones, which interact with transmembrane receptors, have been mentioned above and their functional domains reviewed (e.g. Yamada & Kleinman 1992). At present, the number of integrin ligands is exponentially expanding, in parallel with the number of new integrin heterodimers being discovered. We discuss extracellular ligands and their properties and draw attention to some parameters affecting their interaction with the transmembrane linkers.

Matrix Proteins

Collagens, fibronectin, vitronectin, laminin, and thrombospondin are ECM proteins whose interactions with integrin combinations have been studied in detail (see Haas & Plow 1994 for references). Several cognate motifs in the form of short sequence stretches have been identified as binding domains for transmembrane proteins. In addition to RGD as a consensus-binding sequence for several integrins (see above), YIGSR has been identified in laminin as an important mediator of focal adhesion (Massia et al 1993). Although RGD sequences are usually part of flexible peptide loops, which enable many ECM proteins to interact with several integrins, collagens and laminin interact with other integrins in a conformation-dependent manner (Massia et al 1993, Kühn & Eble 1994, Haas & Plow 1994). Adoption of this conformation may be the first step in the formation of attachment sites on these matrix proteins, but may also be an important trigger of signaling processes associated with focal adhesion sites (see below).

ORGANIZATION AND DYNAMICS OF FOCAL CONTACTS

The main focus of this article is to provide an overview on the molecules participating in the architecture of focal adhesion sites and their interactions. However, it is important to stress that focal contacts are not static but highly dynamic structures that exist for a limited period of time, tightly controlled by a finely balanced equilibrium of their components. Shifting one component

may result in a catastrophic event, leading to the disintegration of the entire structure, whereas reassembling it requires a multitude of cellular reactions to take place.

Aspects of Assembly

Podosomes, adhesive plaques of platelets, and the spear tip–shaped focal contacts of adherent epithelial, endothelial, and fibroblastic cells, all examples of focal adhesions, may differ not only in size and morphological appearance but also in quality. Alpha-actinin isoforms (Waites et al 1992), gelsolin (Wang et al 1984), and MARCKS (Aderem 1992) have already been mentioned as being associated with only some types of focal adhesions, but there may be other proteins that are specifically associated with certain focal adhesion types.

Focal contacts also may be subject to maturation and thus contain subsets of components that differ at the end of their life span from those present at the beginning. Growth of a focal adhesion site may start with the plasma membrane contacting the matrix at the periphery of a fibroblast leading lamella or the activation of platelets by an agonist, which then leads to the reorganization or activation of integrins. Such reorganization or activation may be concomitant with or induced by conformational changes in ECM motifs, in analogy to an induced fit (Massia et al 1993, Haas & Plow 1994, Kühn & Eble 1994). Integrin-induced signaling may then start by triggering a multitude of cascades, leading to phosphorylation (or dephosphorylation) of individual cytoskeletal components. Such modulation may target selected proteins initially to the plasma membrane, as shown for MARCKS (Aderem 1992), the Src kinase (Kaplan et al 1994), phosphokinase C-δ, FAK, paxillin, vinculin, and talin (Bertagnolli et al 1993, Barry & Critchley 1994). In addition to recruitment of preexisting filaments, actin polymerization could take place at these sites, induced by receptor kinase activation, phospholipase Cγ1 activation, and subsequent release of profilin from its membrane-bound complex (Goldschmidt-Clermont et al 1990, Furman et al 1993, Sohn & Goldschmidt-Clermont 1994). Control mechanisms may involve additional polyphosphoinositide-regulating factors, as well as rho and VASP/CAP-like proteins (Heldman & Goldschmidt-Clermont 1993, Hall 1994, Horstrup et al 1994, Nobes et al 1995; cf Figure 5). Elegant microscopic studies on the assembly of nascent focal adhesions from actin-rich precursors in fibroblasts have shown that on the cytoplasmic face, talin is an early structural component collected into F-actin-rich foci, preceding the visual accumulation of vinculin (De Pasquale & Izzard 1987, 1991; Izzard 1988). A conformational change in vinculin, by an as yet unknown signal (see Gilmore & Burridge 1995), would expose its talin-, α-actinin-, and F-actin-binding sites (Menkel et al 1994, Kroemker et al 1994, Johnson & Craig 1994, 1995). Vinculin would then be concentrated in these nascent foci (David-Pfeuty 1985) by the presence of

several high-affinity binding sites on talin and by self-association of vinculin. Alpha-actinin could enforce these structures by linking either vinculin/talin or F-actin to the β_1 subunit of the integrin complex. Such processes could induce the collection and packing of the terminal portions of microfilaments into the focal contacts of adhesive cells or platelets.

Endothelial cells have been shown to develop focal contacts enriched for integrin β_1, talin, α-actinin, vinculin, and paxillin within 15 min, after contact with ECM-coated beads (Plopper & Ingber 1993). Thus several alterations of individual components are already seen in the early stages of contact formation: modulation of the phosphorylation state, conformational changes, exposure, and selective usage of ligand binding domains.

Intensive crosstalk between the different elements at the cytoplasmic side is needed for assembly and maintenance of the focal contact. Principal mechanisms comprise the following: (*a*) SH2 domains, contained in many structural and regulatory proteins, mediate binding to a wealth of components that are tyrosine-phosphorylated as an early event in focal contact formation (Romer et al 1992, 1994). The importance of phosphotyrosinylation is demonstrated in studies where the normal phosphotyrosine content has been experimentally manipulated. Interference with tyrosine kinases prevents focal adhesion assembly (Romer et al 1992, 1994), whereas inhibition of the normal phosphotyrosine turnover by phosphatase inhibitors leads to a dramatic increase in number and size of focal contacts (Volberg et al 1991, 1992; Barry & Critchley 1994). (*b*) Proline-rich motifs, either in the form of SH3-binding sequences, or in the context of insufficiently characterized frames, are involved in such docking phenomena between many cytoskeletal proteins in nascent focal adhesion sites. (*c*) The LIM domains, previously recognized as an essential component directing the interaction of transcription factors, have now been found in an increasing number of cytoskeletal elements of the focal contact.

Maturation Processes

Further maturation might involve selective protein synthesis, exchange of early components for others, and reinforcement of structural bonds by the addition of stabilizing elements. Vinculin and α-actinin syntheses are stimulated by cellular contact with the ECM (Ungar et al 1986, Bendori et al 1987, Glück et al 1992). An exchange of fibronectin-binding integrins for vitronectin receptors during the maturation of focal contacts in endothelial cells has been reported (Burridge et al 1988), and microtubules, as well as intermediate filaments, are associated with the large adhesion areas of quiescent fibroblasts (Geiger et al 1984, Bershadsky et al 1987, Rinnerthaler et al 1988). The finding that many cytoskeletal components, e.g. talin, vinculin, and α-actinin, directly interact with the acidic phospholipids in the plasma membrane (reviewed in Isenberg 1991) suggests that such interactions participate in stabilizing the

final, geometric arrangement of the structure. On the outside of the cell, the focal adhesion structure is frequently stabilized by heparan sulfate proteoglycans interacting with ECM proteins (Singer et al 1987).

Dynamics of Focal Contacts

Unlike the focal adhesion-like structures developed by platelets and smooth muscle, the focal contact sites of cultivated cells are subject to rapid assembly and disassembly. Entry into mitosis or stimulation of locomotor activity leads to rapid breakdown of large, mature contacts in quiescent fibroblast cell-matrix sites, followed by disruption of stress fibers and a rounding up of the cells upon release from their substratum. These events can be mimicked by microinjecting specific antibodies against structural components, e.g. against vinculin (Westmeyer et al 1990) or talin (Nuckolls et al 1992). In the unperturbed cell, disassembly may be induced by a reversal of virtually all assembly mechanisms described above, but specific data are not available to verify this. In addition, proteases similar to the Ca^{2+}-dependent protease calpain II may play a role in degrading individual components upon a specific signal (Beckerle et al 1987). An important factor in the dynamic nature of the focal contact site is the fact that all components seem to interact with low affinities that usually do not exceed K_d values of 10^{-6} M. The only exceptions so far known are the vinculin-talin complex and the vinculin-paxillin complex with K_d values of 10^{-8} and 6×10^{-8} M, respectively (Burridge & Mangeat 1984, Turner et al 1990, Gilmore et al 1993). Thus by employing a multitude of components that interact with modest affinities, the cell gains a high degree of versatility and velocity in assembly/disassembly of focal adhesion sites.

FUTURE PERSPECTIVES: FOCAL CONTACTS AS MULTIFUNCTIONAL STRUCTURES

Initially, focal contacts were considered as specifically needed for attachment, spreading, and locomotor activity (in the form of podosomes). More recently, however, we have learned that these complex functions are only the tip of the iceberg. Cell-matrix adhesion sites are now known to be "hot spots" for cell signaling. A cascade of several signal transduction pathways is turned on by the initial contact between transmembrane proteins and extracellular ligands (Damsky & Werb 1992, Juliano & Haskill 1993, Sastry & Horwitz 1993). The diversity of these pathways, which results from their number and frequent branching points, ascertains that signals can be sent virtually into every compartment of the cell including the nucleus. These signaling pathways may also act in the opposite direction: Engagement of the cytoplasmic domains of the transmembrane molecules by cytoskeletal elements can be used for "inside-out" signaling, conveying cellular information to the extracellular milieu (re-

viewed in Hynes 1992, Ginsberg et al 1992, Juliano & Haskill 1993, Sastry & Horwitz 1993, Williams et al 1994). When viewed this way, the term focal contact can be interpreted as a structural unit directing the flow of information that is the basis for the communication between cells and their environment. In this sense, focal adhesions are the computer chips of animal cells.

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